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*Short Communications*

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## **Mushroom Body Development in Nymphalid Butterflies: A Correlate of Learning?**

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*Accepted 3 May 1988; revised 9 September 1988*

**KEY WORDS:** development; learning; mushroom body; Nymphalidae.

Among the more complex features of the arthropod brain are the paired, densely staining structures in the protocerebrum known as corpora pedunculata or, more graphically, as mushroom bodies. They are integrated closely with the deutocerebrum and optic lobes and their major function is believed to be the synthesis of olfactory information (Happ and Eickwort, 1984). However, the mushroom bodies also have long been associated with behavioral complexity [e.g., "organs of intelligence" (Wheeler, 1910)]. Dujardin (1850; as discussed by Wheeler, 1910) noted their greater development in social species of Hymenoptera. Within a single species of ant, the size of these bodies can be correlated with the breadth of the behavioral repertoire, i.e., vestigial in males, larger in queens and still bigger yet in the brains of the sophisticated workers (Forel, 1874; such caste polymorpha are not universal; see Wheeler, 1910). While this pattern of development also is consistent with the use of more diverse chemical signals by workers, subsequent research has supported an "intelligence" component in insect mushroom bodies, more specifically, their importance in learning (Happ and Eickwort, 1984, Erber *et al.*, 1987). During episodes of shock avoidance learning in orthopteroids, RNA synthesis increases disproportionately in mushroom bodies. The resulting protein synthesis is indicative of the neural growth and revision that are the physical basis of learning (reviewed by Punzo, 1985). Interestingly, *Drosophila melanogaster* (Meigen) kept alone in simple environments have smaller mushroom bodies than those living with various odor sources, plants, and other flies (Technau, 1984). The morphology of mushroom body neurons differs between foraging bees and newly emerged sisters (Coss *et*

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al., 1980). Olfactory learning is impaired in *D. melanogaster* mutants with structural defects in the mushroom bodies (Heisenberg *et al.*, 1985), although other behaviors are unaffected (Heisenberg, 1980). The transfer of information from short- to long-term memory in honey bees requires the mushroom bodies (Erber *et al.*, 1980) and memories (learned feeding times) have been transplanted by placing "experienced" mushroom bodies into the heads of naive bees (Martin *et al.*, 1978).

The following describes substantial variation in mushroom body development among seven species of Nymphalidae. Two possible explanations for this variation, differences in amount of sensory input and differing abilities to learn, are considered. This is done by attempting to correlate mushroom body size both with the sizes of sensory structures and with behaviors that include, or are suggestive of, learning.

An entire brain of a male and a female of the following butterflies were examined: *Heliconius charitonius* (L.), *Dryas iulia* (Fab.), *Agraulis vanillae* (L.), *Siproeta stelenes* (L.), *Marpesia petreus* (Cramer), *Euptoieta claudia* (Cramer) (two males and one female), and *Junonia coenia* (Cramer) (two females). Additional specimens were sectioned and stained with either Mallory's triple stain or a silver stain (see Loots *et al.*, 1979) but not measured, largely because of occasional missing or malformed sections that made quantification difficult. After inspection of all these preparations, the measured individuals appeared typical.

The brain was sectioned in 8- $\mu$ m slices but only every other section was measured, so that in practice the brain was examined in 16- $\mu$ m slices. This typically resulted in 80–110 measured sections per hemisphere. Area measurements were made on a computerized digitizer via a video image magnified 150 $\times$ . Note that there is considerable shrinkage of brain tissue in fixatives, so that sizes are underestimated and the measurements are most useful in relative comparisons.

The gross morphology of the brain was similar across species, with sagittal sections being the easiest to interpret. Beginning with the most distal portions of the brain, the following areas were measured: optic lobe, mushroom body [measured in three regions, the calyx (the large cap of the mushroom), the stalk, and the combination of the lower  $\alpha$  and  $\beta$  lobes], antennal nerves and lobe (the long striated antennal nerves that are closely associated with the brain and the antennal lobe were measured as a single entity), subesophageal ganglion, and major brain (the brain outside of the antennal structures, optic lobe, and subesophageal ganglion). Data for total brain volumes are doublings of measurements of one hemisphere.

All insects were captured in Florida (Alachua, Dade, and Monroe Counties). In most cases the body, without wings or head, was preserved separately in 70% ethanol, dried at 100°C for 24 h, and weighed. If the body was not available, a mean value from other conspecifics was ascribed to it.

The total and major brain per gram of dry body weight has a considerable range. The highest values occur in *H. charitonius* and *E. claudia* (Table I; see Gilbert, 1975). The proportion of the brain occupied by the optic lobes is similar across species, with the smallest proportion (*H. charitonius*, 0.73) being 87% of the largest (*A. vanillae*, 0.84; *S. stelenes*, 0.84) (Table II). The antennal lobes range from 2.2% of the total brain (*D. iulia*) to 1.3% (*E. claudia*) (Table II). The range of relative mushroom body sizes is considerably greater and spans an order of magnitude (4.2% in *H. charitonius* to 0.3% in *S. stelenes*; Table II). Much of this variance is due to the very large mushroom bodies of *H. charitonius*, which are nearly three times as extensive as the next most endowed species, *E. claudia*.

**Table I.** The Size, Absolute and Relative, of the Brain in the Seven Species of Nymphalidae

	Total brain volume ( $\mu\text{m}^3$ )	Total brain ( $\mu\text{m}^3$ ) per g body weight	Major brain ( $\mu\text{m}^3$ ) per g body weight
<i>Heliconius charitonius</i>			
Male	1.1 <sup>9</sup>	6.4 <sup>10</sup>	1.4 <sup>10</sup>
Female	1.3 <sup>9</sup>	6.6 <sup>10</sup>	1.5 <sup>10</sup>
<i>Dryas iulia</i>			
Male	1.5 <sup>9</sup>	3.8 <sup>10</sup>	5.6 <sup>9</sup>
Female	1.5 <sup>9</sup>	5.0 <sup>10</sup>	8.4 <sup>9</sup>
<i>Agraulis vanillae</i>			
Male	1.0 <sup>9</sup>	2.2 <sup>10</sup>	3.6 <sup>9</sup>
Female	1.1 <sup>9</sup>	3.2 <sup>10</sup>	4.4 <sup>9</sup>
<i>Siproeta stelenes</i>			
Male	1.2 <sup>9</sup>	3.8 <sup>10</sup>	5.2 <sup>9</sup>
Female	1.1 <sup>9</sup>	1.8 <sup>10</sup>	3.0 <sup>9</sup>
<i>Marpesia petreus</i>			
Male	—	—	4.2 <sup>9</sup>
Female	1.2 <sup>9</sup>	4.8 <sup>10</sup>	8.4 <sup>9</sup>
<i>Juonia coenia</i>			
Female	—	—	2.6 <sup>9</sup>
Female	9.6 <sup>8</sup>	1.5 <sup>10</sup>	1.1 <sup>9</sup>
<i>Euptoieta claudia</i>			
Male	9.6 <sup>8</sup>	4.8 <sup>10</sup>	8.6 <sup>9</sup>
Male	1.1 <sup>9</sup>	6.6 <sup>10</sup>	1.2 <sup>10</sup>
Female	1.0 <sup>9</sup>	6.0 <sup>10</sup>	9.5 <sup>9</sup>

**Table II.** The Proportion of the Total Brain Occupied by Sensory Structures and the Mushroom Bodies and the Ratio of the Mushroom Body Volume to the Volume of the Olfactory Lobes

	Percentage of brain occupied by			Ratio of volume of mushroom body to volume of olfactory lobe
	Optic lobes	Antennal lobes	Mushroom bodies	
<i>Heliconius charitonius</i>				
Male	73	1.6	4.2	2.6
Female	75	1.6	4.2	2.6
<i>Dryas iulia</i>				
Male	82	1.7	0.7	0.4
Female	79	2.2	0.9	0.4
<i>Agraulis vanillae</i>				
Male	79	1.0	0.8	0.8
Female	84	1.5	1.1	0.7
<i>Siproeta stelenes</i>				
Male	84	1.4	0.4	0.3
Female	78	1.7	0.3	0.2
<i>Marpesia petreus</i>				
Male	—	—	—	—
Female	79	1.4	0.6	0.4
<i>Juonia coenia</i>				
Male	—	—	—	—
Female	82	1.6	0.5	0.3
<i>Euptoieta claudia</i>				
Male	79	1.6	1.4	0.9
Male	77	1.8	1.3	0.7
Female	81	1.3	0.8	0.6

The mushroom bodies and sensory structures of the brain, particularly the olfactory lobe, do not always share a corresponding development. There is an order-of-magnitude range in the ratio of the mushroom body volume to the volume of the olfactory lobes (Table II).

There is no evidence of an allometric relationship between brain and body size (see Jerison, 1973; Rensch, 1956). Only in the nonheliconiine nymphalid

is there a possibility of a negative allometry. Major brain and mushroom body sizes are inversely ranked with mean body weight (Table III). However, the big mushroom bodies of the smallest species, *E. claudia*, not only are proportionally larger, but also are absolutely larger, making it unlikely that allometry accounts for their greater relative size.

The multiple functions of the mushroom bodies can distort broad phylogenetic comparisons between the extent of mushroom body development and the behavioral complexity/flexibility. As early as 1910, Wheeler expressed his skepticism that mushroom bodies were the arthropod "analogue of the human forebrain" by noting their great size in the horseshoe crab (*Limulus*), an animal "that has had ages in which to acquire a high physical endowment" but that "shows no sign of having profited by its opportunities." To make a similar point, Happ and Eickwort (1984) noted that like "smart" social insects, "dumber" but highly olfactory moths have large mushroom bodies, while those of the visually sensitive Diptera and Odonata are small. However, such comparisons of behavior and mushroom body size might be more profitable when made between closely related animals that share similar sensory environments. Among *Formica rufa* L. workers, those more efficient in maze learning have broader mushroom body calyxes (Bernstein and Bernstein, 1969).

In the Nymphalidae there is variance in relative mushroom body size that is not correlated with differences in olfactory and optic lobe size. If similar lobe sizes reflect similar amounts and varieties of sensory input, then it seems possible that this range in mushroom body size is due to differing abilities to learn. *H. charitonius* is a passion flower (*Passiflora*) specialist, like its heliconiini relatives *D. iulia* and *A. vanillae*. All three have similar optic and olfactory lobe development relative to other brain constituents, but the mushroom bodies of *H. charitonius* are about four times as large. *Heliconius* species are long-lived pollen feeders that trapline within home ranges during the day and aggregate in communal roosts at night (Gilbert, 1972, 1975; Waller and Gilbert, 1982). Compared to other members of their tribe, they typically occur in more forested

**Table III.** The Mean Dry Weight (g), Relative Mushroom Body Size ( $\mu\text{m}^3/\text{g}$ ) and Volume ( $\mu\text{m}^3$ ) of Mushroom Bodies of Four Species of Nymphalinae (Species Are in Order of Weight)

	Mean body weight (g)	Mushroom body volume ( $\mu\text{m}^3$ ) per g body weight	Volume of mushroom body ( $\mu\text{m}^3$ )
<i>Euptoieta claudia</i>	0.0182	6.8 <sup>8</sup>	4.2 <sup>6</sup>
<i>Marpesia petreus</i>	0.0310	2.0 <sup>8</sup>	5.7 <sup>6</sup>
<i>Siproeta stelenes</i>	0.0481	1.0 <sup>8</sup>	5.3 <sup>6</sup>
<i>Juonia coenia</i>	0.0538	1.0 <sup>8</sup>	1.3 <sup>7</sup>

habitats containing scattered resources and this may be responsible for their memorizing the locations of food sources and conspecifics (Turner, 1981). Their reputation for learning and other relatively sophisticated behaviors led Turner (1981) to predict "some elaboration of the nervous system" in comparison with other butterflies (see also Gilbert, 1975). One obviously elaborated structure in the brain is the large mushroom bodies.

Another, if more minor, peak in mushroom body development occurs in *E. claudia*. The mushroom bodies are noticeably larger than those of the other nonheliconiini nymphalines and, at least in the male dissections, larger than the heliconiins, *D. iulia* and *A. vanillae*. The significance of these large bodies is unknown. Learning the locations of particular, rare larval hosts, as in *Heliconius* spp., is unlikely since they feed on plants from a large number of families (Pyle, 1981; but see the discussion of learning and generalism by Vet, 1984, Greenburg, 1985, Daly *et al.*, 1982). It is possible that the distribution of adult food might contribute to the evolution of learning even more than the distribution of oviposition sites (see Gilbert, 1984, personal communication). It is not known if *E. claudia* performs any unusual foraging tactic such as traplining. Again, an alternative might be sensitivity to chemicals or a complicated communication that is not reflected in increased olfactory lobe development.

Complex objects such as nymphalid mushroom bodies are likely to have complex phylogenies and the simple correlations offered here probably are insufficient for complete explanations. The size range of these structures does suggest different kinds or degrees of behaviors and/or sensory abilities, so behaviorists might take these differing brain developments into account when observing the discussed species.

## ACKNOWLEDGMENTS

Pat Carlyle taught me to section and stain and provided me with the apparatus and chemicals. Roger Reep made measurement of the brain sections practical by giving me access to his computer and microscope. M. S. Mayer helped by finding a number of obscure references. Larry Gilbert made some important criticisms of early drafts.

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